

IN THE CLAIMS

1. **(CURRENTLY AMENDED)** A DNA expression construct comprising, in 5' to 3' order: a promoter, the promoter operationally linked to a secretion signal sequence, the secretion signal sequence operationally-linked to a DNA sequence encoding a proteolytic tryptase having an active site mutation at an amino acid position selected from positions 44, 91, and 194 of the proteolytic tryptase as shown in Fig. 1, which positions correspond to residues 48, 95, and 198, respectively, of SEQ. ID. NOS: 6, 9, 21, 23, 25, 27, 37, 39, 41, and 43, and wherein the expression construct drives expression of a mature proteolytic tryptase that lacks enzymatic activity in eukaryotic host cells transformed to contain the expression construct, the lack of enzymatic activity being due to the active site mutation.
2. **(CURRENTLY AMENDED)** The DNA expression construct according to Claim 1, wherein the DNA sequence encoding the proteolytic tryptase having an active site mutation encodes β -H skin tryptase.
3. **(CURRENTLY AMENDED)** The DNA expression construct according to Claim 1, wherein the DNA sequence encoding the proteolytic tryptase having an active site mutation encodes β -H lung tryptase.
4. **(ORIGINAL)** The DNA expression construct according to Claim 1, wherein the DNA sequence encoding the proteolytic tryptase having an active site mutation encodes a human proteolytic tryptase.
5. **(PREVIOUSLY PRESENTED)** The DNA expression construct according to Claim 1, wherein the active site mutation changes a native amino acid to a non-charged amino acid.

6. (PREVIOUSLY PRESENTED) The DNA expression construct according to Claim 5, wherein the active site mutation changes a native amino acid to an alanine.

7. (CURRENTLY AMENDED) ~~The DNA expression construct according to Claim 1, A DNA expression construct comprising, in 5' to 3' order: a promoter, the promoter operationally linked to a secretion signal sequence, the secretion signal sequence operationally-linked to a DNA sequence encoding a proteolytic trypsinase having an active site mutation at an amino acid position selected from positions 44, 91, and 194 of the proteolytic trypsinase as shown in Fig. 1 (which positions correspond to residues 48, 95, and 198, respectively, of SEQ. ID. NOS: 6, 9, 21, 23, 25, 27, 37, 39, 41, and 43), wherein the DNA sequence encoding the proteolytic trypsinase having an active site mutation has is a DNA sequence selected from the group consisting of SEQ. ID. NO. 20, SEQ. ID. NO. 22, SEQ. ID. NO. 24, SEQ. ID. NO. 26, SEQ. ID. NO. 36, SEQ. ID. NO. 38, SEQ. ID. NO. 40, and SEQ. ID. NO. 42, and wherein the expression construct drives expression of a mature proteolytic trypsinase that lacks enzymatic activity in eukaryotic host cells transformed to contain the expression construct, the lack of enzymatic activity being due to the active site mutation.~~

8. (CURRENTLY AMENDED) ~~The DNA expression construct according to Claim 7, A DNA expression construct comprising, in 5' to 3' order: a promoter, the promoter operationally linked to a secretion signal sequence, the secretion signal sequence operationally-linked to a DNA sequence encoding a proteolytic trypsinase having an active site mutation at an amino acid position selected from positions 44, 91, and 194 of the proteolytic trypsinase as shown in Fig. 1 (which positions correspond to residues 48, 95, and 198, respectively, of SEQ. ID. NOS: 6, 9, 21, 23, 25, 27, 37, 39, 41, and 43), and wherein the proteolytic trypsinase has an amino acid sequence selected from the group consisting of SEQ. ID. NO. 21, SEQ. ID. NO. 23, SEQ. ID. NO. 25, SEQ. ID. NO. 27, SEQ. ID. NO.~~

37, SEQ. ID. NO. 39, SEQ. ID. NO. 41, and SEQ. ID. NO. 43, and further
wherein the expression construct drives expression of a mature proteolytic
tryptase that lacks enzymatic activity in eukaryotic host cells transformed to
contain the expression construct, the lack of enzymatic activity being due to the
active site mutation.

9. **(ORIGINAL)** The DNA expression construct according to Claim 1, wherein the secretion signal sequence encodes a KEX2 cleavage site.
10. **(ORIGINAL)** The DNA expression construct according to Claim 1, wherein the secretion signal sequence includes a 3' terminus encoding amino acid residues Leu-Glu-Lys-Arg.
11. **(ORIGINAL)** The DNA expression construct according to Claim 1, wherein the promoter is a constitutive promoter.
12. **(ORIGINAL)** The DNA expression construct according to Claim 1, wherein the promoter is an inducible promoter.
13. **(ORIGINAL)** A DNA expression construct comprising, in 5' to 3' order: a promoter selected from the group consisting of *AOXI*, *GAP*, *MOX*, *FMD*, *ADH*, *LAC4*, *XPR2*, *LEU2*, *GAM1*, *PGK1*, *GAL7*, *GADPH*, *CYC1*, and *CUP1*, the promoter operationally linked to a secretion signal sequence, the secretion signal sequence operationally-linked to a DNA sequence encoding proteolytic tryptase having an active site mutation, the DNA sequence operationally linked to a terminator sequence.

14. **(CURRENTLY AMENDED)** The DNA expression construct according to Claim 13, wherein the DNA sequence encoding the proteolytic tryptase encodes **β -I skin** tryptase.
15. **(CURRENTLY AMENDED)** The DNA expression construct according to Claim 13, wherein the DNA sequence encoding the proteolytic tryptase encodes **β -H lung** tryptase.
16. **(ORIGINAL)** The DNA expression construct according to Claim 13, wherein the DNA sequence encoding the proteolytic tryptase having an active site mutation encodes a human proteolytic tryptase.
17. **(CURRENTLY AMENDED)** ~~The DNA expression construct according to Claim 13~~ **A DNA expression construct comprising, in 5' to 3' order: a promoter selected from the group consisting of *AOX1*, *GAP*, *MOX*, *FMD*, *ADH*, *LAC4*, *XPR2*, *LEU2*, *GAM1*, *PGK1*, *GAL7*, *GADPH*, *CYC1*, and *CUP1*, the promoter operationally linked to a secretion signal sequence, the secretion signal sequence operationally-linked to a DNA sequence encoding proteolytic tryptase having an active site mutation, the DNA sequence operationally linked to a terminator sequence**, wherein the DNA sequence encoding the proteolytic tryptase having an active site mutation **has is** a DNA sequence selected from the group consisting of SEQ. ID. NO. 20, SEQ. ID. NO. 22, SEQ. ID. NO. 24, SEQ. ID. NO. 26, SEQ. ID. NO. 36, SEQ. ID. NO. 38, SEQ. ID. NO. 40, and SEQ. ID. NO. 42.
18. **(CURRENTLY AMENDED)** ~~The DNA expression construct according to Claim 13~~ **A DNA expression construct comprising, in 5' to 3' order: a promoter selected from the group consisting of *AOX1*, *GAP*, *MOX*, *FMD*, *ADH*, *LAC4*, *XPR2*, *LEU2*, *GAM1*, *PGK1*, *GAL7*, *GADPH*, *CYC1*, and *CUP1*, the promoter operationally linked to a secretion signal sequence, the secretion signal**

sequence operationally-linked to a DNA sequence encoding proteolytic trypsinase
having an active site mutation, the DNA sequence operationally linked to a
terminator sequence, wherein the proteolytic trypsinase has DNA sequence
encoding the proteolytic trypsinase having an active site mutation encodes an
amino acid sequence selected from the group consisting of SEQ. ID. NO. 21, SEQ.
ID. NO. 23, SEQ. ID. NO. 25, SEQ. ID. NO. 27, SEQ. ID. NO. 37, SEQ. ID.
NO. 39, SEQ. ID. NO. 41, and SEQ. ID. NO. 43.

19. **(ORIGINAL)** The DNA expression construct according to Claim 13, wherein the secretion signal sequence encodes a KEX2 cleavage site.
20. **(CURRENTLY AMENDED)** A method of producing ~~an active site a~~ mutation of proteolytic trypsinases comprising transforming a eukaryotic host cell with an expression construct according to Claim 1, wherein the mutation causes the eukaryotic host cell expresses to express enzymatically-inactive proteolytic trypsinase.
21. **(ORIGINAL)** The method according to Claim 20, wherein a yeast host cell is transformed.
22. **(CURRENTLY AMENDED)** The method according to Claim 21, wherein ~~a the~~ transformed yeast host cell is of the genus *Pichia* ~~is transformed~~.
23. **(CURRENTLY AMENDED)** The method according to Claim 22, wherein ~~a the~~ transformed yeast host cell is *Pichia pastoris* ~~host cell is~~ transformed.
24. **(CURRENTLY AMENDED)** The method according to Claim 23, wherein ~~a the~~ transformed yeast host cell having has the characteristics of *Pichia pastoris* ATCC 20864 or *Pichia pastoris* strain KM71 ~~is transformed~~.

25. **(ORIGINAL)** The method according to Claim 20, further comprising isolating the enzymatically-inactive proteolytic tryptase produced.

Claims 26-33 (CANCELED).

34. **(CURRENTLY AMENDED)** A genetically-engineered eukaryotic cell which expresses enzymatically-inactive proteolytic tryptase **comprising a** wherein the eukaryotic host cell **is** transformed to contain and express an expression construct according to Claim 1.

35. **(ORIGINAL)** The genetically engineered eukaryotic cell of Claim 34, wherein the eukaryotic cell is a yeast cell.

36. **(ORIGINAL)** The genetically-engineered eukaryotic cell of Claim 35, wherein the yeast cell is of the genus *Pichia*.

37. **(CURRENTLY AMENDED)** A genetically-engineered eukaryotic cell which expresses enzymatically-inactive proteolytic tryptase **comprising a** wherein the eukaryotic host cell **is** transformed to contain and express an expression construct according to Claim 13.

Claims 38-40 (CANCELED).

41. **(PREVIOUSLY PRESENTED)** A DNA expression construct comprising, in 5' to 3' order: a promoter, the promoter operationally linked to a secretion signal sequence, the secretion signal sequence operationally-linked to a DNA sequence encoding proteolytic tryptase, wherein the expression construct drives the expression of mature proteolytic tryptase that has enzymatic activity in eukaryotic host cells transformed to contain the expression construct.

42. (ORIGINAL) The DNA expression construct of Claim 41, wherein the DNA sequence encoding the proteolytic tryptase encodes a human proteolytic tryptase.

43. (CURRENTLY AMENDED) ~~The DNA expression construct of Claim 42 A DNA expression construct comprising, in 5' to 3' order: a promoter, the promoter operationally linked to a secretion signal sequence, the secretion signal sequence operationally-linked to a DNA sequence encoding proteolytic tryptase,~~ wherein the DNA sequence comprises SEQ. ID. NO. 8, and wherein the expression construct drives the expression of mature β -H lung tryptase that has enzymatic activity in hosts transformed to contain the expression construct.

44. (CURRENTLY AMENDED) A method of producing enzymatically-active β -H lung tryptase comprising transforming a eukaryotic host cell with an expression construct according to Claim 43, wherein the host cell expresses enzymatically-active β -H lung tryptase.

45. (CURRENTLY AMENDED) The method according to Claim 44, further comprising isolating the enzymatically-inactive enzymatically-active proteolytic tryptase produced.

Claims 46-53 (CANCELED).

54. (ORIGINAL) The method of Claim 44, wherein a yeast host is transformed.

55. (CURRENTLY AMENDED) The method of Claim 54, wherein the transformed yeast host is a *Pichia* host is transformed.

56. (CURRENTLY AMENDED) A genetically-engineered eukaryotic cell which expresses enzymatically-active β -H lung tryptase comprising a wherein the eukaryotic host cell is transformed to contain and express an expression construct according to Claim 43.

57. (ORIGINAL) The genetically engineered eukaryotic cell of claim 56, wherein the eukaryotic cell is a yeast cell.

58. (ORIGINAL) The genetically-engineered eukaryotic cell of Claim 57, wherein the yeast cell is a *Pichia* cell.

Claims 59-61 (previously canceled).

62. (CANCEL) ~~A DNA expression construct comprising, in 5' to 3' order: a promoter selected from the group consisting of *AOX1*, *GAP*, *MOX*, *FMD*, *ADH*, *LAC4*, *XPR2*, *LEU2*, *GAM1*, *PGK1*, *GAL7*, *GADPH*, *CYC1*, and *CUP1*, the promoter operationally linked to a secretion signal sequence, the secretion signal sequence operationally linked to a DNA sequence selected from the group consisting of SEQ. ID. NO. 20, SEQ. ID. NO. 22, SEQ. ID. NO. 24, SEQ. ID. NO. 26, SEQ. ID. NO. 36, SEQ. ID. NO. 38, SEQ. ID. NO. 40, and SEQ. ID. NO. 42, the DNA sequence operationally linked to a terminator sequence.~~

63. (CANCEL) ~~The DNA expression construct according to Claim 13, wherein the DNA sequence encodes an amino acid sequence selected from the group consisting of SEQ. ID. NO. 21, SEQ. ID. NO. 23, SEQ. ID. NO. 25, SEQ. ID. NO. 27, SEQ. ID. NO. 37, SEQ. ID. NO. 39, SEQ. ID. NO. 41, and SEQ. ID. NO. 43.~~